Black Kernel Rot of Corn in Thailand*

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Abstract

Black kernel rot of corn has been frequently observed in corn fields and farmer's storage facilities in Central Thailand. *Botryodiplodia theobromae* was isolated from black kernels. Inoculation experiments with *B. theobromae* showed that the fungus readily infected corn ears and kernels at the milky stage.

Introduction

Botryodiplodia theobromae Pat. has been known as a fungus occurring over wide areas in the tropics. The fungus attacks many tropical crops and causes dieback, root rot, rot or decay of various fruits (avocado, banana, citrus, mango, papaya, etc.), rubber,cacao, tea, cotton, yams, cassava and sweet potato[1,3,4,5,6,8]. The fungus is also the causal organism of sap-stain or blue-stain of tropical hardwoods[1,5,6]. Recently, *B. theobromae* has been isolated from dieback stem of pear in Tokushima Pref., Japan[9]. Furthermore, this fungus is important as a cause of postharvest diseases of many crops, especially storage rot of tropical fruits[3,4,5,6].

Del Rosario[2] first reported black kernel rot of corn caused by *B. theobromae*[7]. In 1983, we observed frequently black kernel rot of corn and collected samples in Central Thailand. We therefore carried out isolation of the fungus from samples of black kernels and examined its morphology. We also made inoculation experiments with the fungus onto corn ears.

Observations

In 1983, we were able to observe and collect many corn ears with black kernels infected with *B. theobromae* in fields and farmer's storage facilities in Saraburi, Lopburi and Phetchaboon Provinces, Thailand (Figs. 1–4). The color of the cob and the base of the cob infected with the fungus turns black (Figs. 1, 3, 5) and the inner part of the cob usually collapses (Fig. 5). After the cobs are infected, the fungus attacks kernels. During the early stage of infection, kernels show a brown discoloration. As the disease progresses, the kernels become black (Figs. 1, 2, 5), and the inner part of the kernels collapses and is occupied with a mass of mycelia (Fig. 6). Pycnidia of the fungus are sometimes observed on the infected kernels (Fig. 4).

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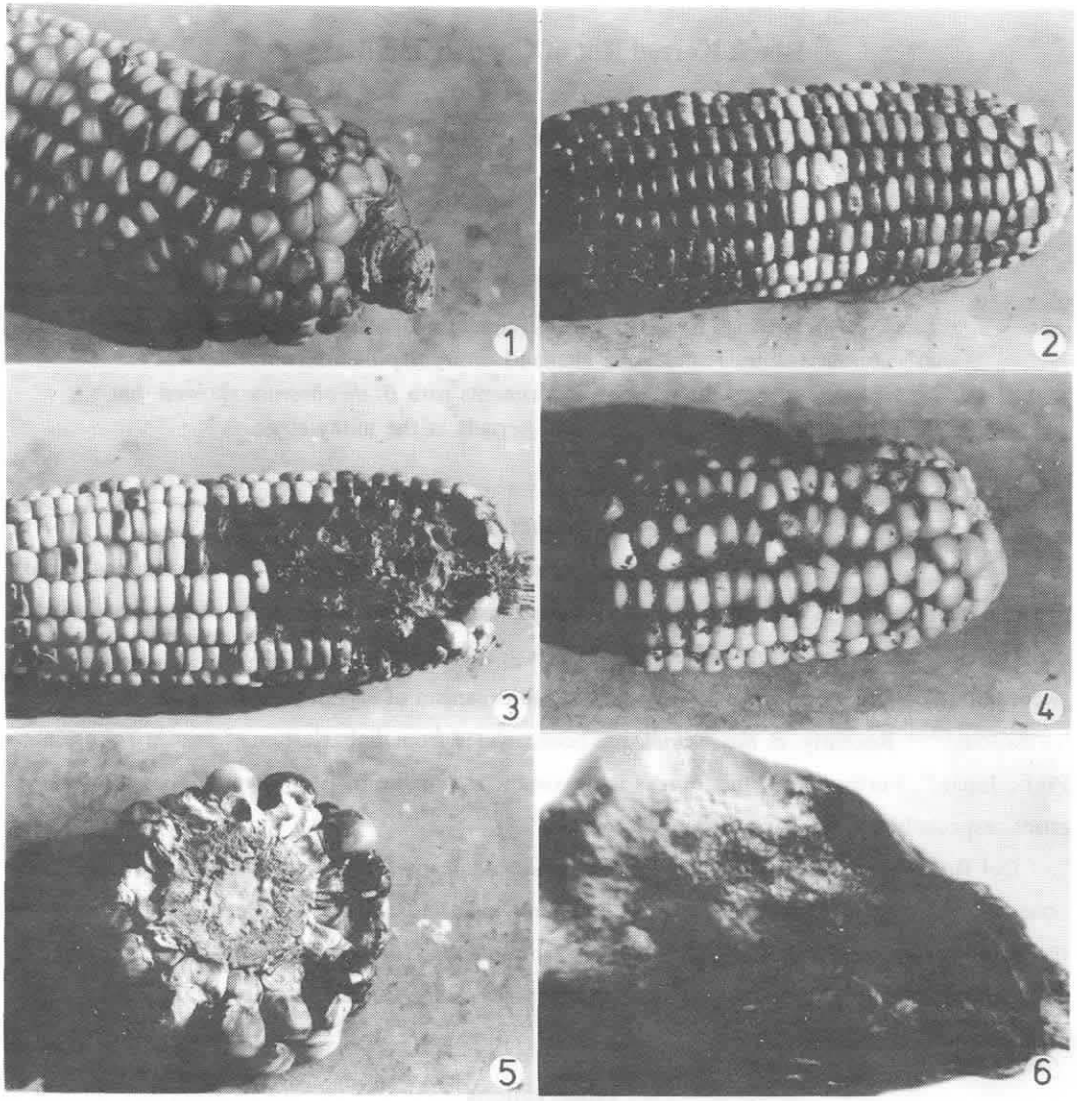


Figs. 1–6. Symptoms of black kernel rot. 1. Base of cob and kernels infected with *Botryodiplodia theobromae*. 2. Heavily infected kernels. 3. Infected cob and kernels. 4. Pycnidia on infected kernels. 5. Inner part of an infected cob. 6. Inner part of an infected kernel.

Isolation of the Fungus

Black kernels collected in corn fields or farmer's storage facilities were put on potato dextrose agar medium(PDA) in petri dishes. After 2 day-incubation at 25–30°C, blackish colonies appeared on the kernels or around them. Pycnidia were sometimes produced in the colonies on the kernels. These colonies were transferred to PDA in petri dishes. The growth of the colonies was extremely fast. The fungus covered PDA in a 9 cm petri dish within 2 days at 25–30°C. The culture of the fungus was maintained on slants of PDA.

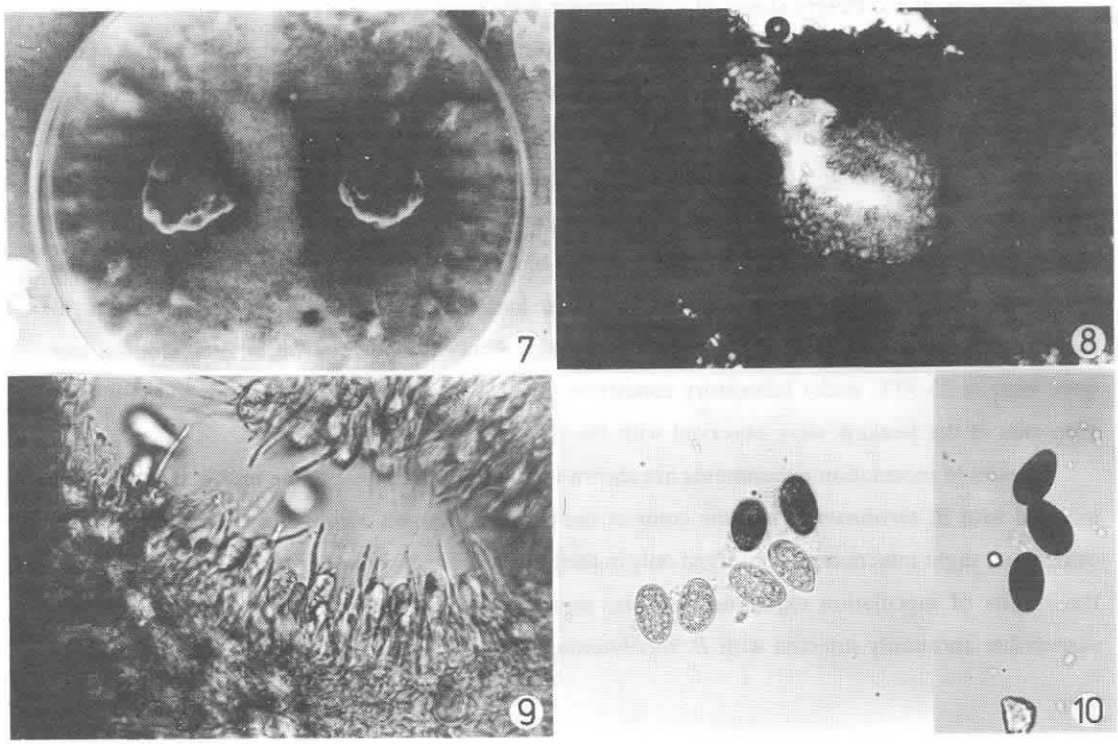
Corn ears collected in fields and farmer's storage facilities were used for determining the frequency of occurrence of the fungus. Samples of corn ears were collected from 32 fields in Lopburi and Saraburi Provinces. Samples were also collected from 11 farmer's storage facilities in Lopburi and Phetchaboon Provinces. After threshing each of the samples, fifty kernels per sample were picked up at random. Five kernels per petri dish were placed on PDA in a 9 cm petri dish. Two or three drops of 20% lactic acid per petri dish were added to PDA to inhibit the growth of bacteria. When the fungus appeared on the surface of the kernels, it was identified under a binocular microscope.

The fungus causing black kernel rot was detected from 13 and 10 samples collected in fields and farmer's storage facilities, respectively. The results showed that the fungus was widespread and frequently detected in corn lesions in Central Thailand. The fungus may be an important limiting factor to corn production in Thailand.

Morphology of the Fungus

Observations on the morphology of the fungus isolated from black kernels were made by light microscopy. A part of the mycelium and hand-sections of pycnidia produced in the stroma were picked up and mounted in a drop of lactophenol solution on glass slides.

Colonies of the fungus on PDA are grayish black with abundant aerial mycelia (Fig. 7). Hyphae



Figs. 7–10. *Botryodiplodia theobromae*. 7. Colonies on PDA. 8. Pycnidium in the stroma produced on infected kernel. 9. Pycnidium. 10. Immature and mature conidia.

are branched, septate and dark brown. Pycnidia are usually produced in the stroma on infected kernels (Fig. 7). Production of pycnidia on PDA seldom occurs under laboratory conditions. Alasoadura[1] reported that light was effective in the production of pycnidia of *B. theobromae*. Pycnidia are single or grouped, ostiolate with paraphyses (Figs. 8, 9). Conidiogenous cells are hyaline, cylindrical, holoblastic and annelidic. Conidia are initially unicellular, thick-walled, hyaline and subovoid to ellipsoid (Fig. 10). Mature conidia are uniseptate, thick-walled, dark brown, longitudinally striate, subovoid to ellipsoid and 20-30 X 10-15 μm in size (Fig. 10). Morphology of the fungus agreed with the descriptions of *B. theobromae*[1,5,6]. Therefore, we identified the fungus as *B. theobromae*.

Inoculation Experiments

We carried out inoculation experiments with *B. theobromae* isolated from the black kernels of corn to examine its pathogenicity to corn ears.

Two black kernels infected with *B. theobromae* were placed on PDA in petri dishes. After incubation for 5-7 days at 25-30°C, abundant pycnidia were produced on the kernels, and colonies grew on the medium. A small amount of sterile distilled water was poured into the petri dishes. After rubbing the surface of the colonies with a painting brush, the suspension was filtered through double layers of gauze. The suspension containing hyphal segments and conidia was used as inoculum.

Corn ears at 4 different stages of development were used for inoculation experiments with *B. theobromae*. In the first stage corn ears at the milky stage were collected from a field of the Maize Project Center at Koktoom, Lopburi Province. In the second stage premature ears were collected at the Center and seed moisture was 29.6%. In the third and the last stages mature ears with a seed moisture of 24.9% and 16.1% respectively were collected. The corn ears were cut about 3 cm in length. Four or six pieces (blocks) of corn ears were prepared per sample. Each of the blocks was put into small beakers (100 ml volume). Half of the blocks in the beakers were kept under moist conditions with wetted filter papers, and the other half under dry conditions. After the fungal suspension had been sprayed on the ear blocks, the beakers were covered with aluminium foil and were kept at 25-30°C under laboratory conditions. About 10 days after inoculation, the blocks of corn ears in the beakers were observed with the naked eye.

Results of inoculation experiments are shown in Table 1. Corn ears at the milky stage were all infected with *B. theobromae*, and the color of the cobs and kernels turned brown to black. In the other ears, slight infection was observed only in the blocks of ears kept under moist conditions. From the results of inoculation experiments it was concluded that the ears at the milky stage were susceptible and easily infected with *B. theobromae*.

Table 1. Result of inoculation experiments with *Botryodiplodia theobromae*

Stage of corn development	Treatment	Replication	Date of inoculation	Infection* Cob	Infection* Kernel
Milky	no	1	Sept. 29, 1983	+	+
		2		+	+
		3		+	+
	wetted	1		+	+
		2		+	+
		3		+	+
Pre-mature (Seed moisture =29.6%)	no	1	Sept. 29, 1983	-	-
		2		-	-
	wetted	1		+	+
		2		-	-
Mature (Seed moisture =24.9%)	no	1	Sept. 29, 1983	-	-
		2		-	-
	wetted	1		+	-
		2		+	+
Mature (Seed moisture =16.1%)	no	1	Sept. 23, 1983	-	-
		2		-	-
	wetted	1		+	-
		2		-	-

* +: infected, -: not infected

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